although with lower intensities. Purified PSII particles exhibit an essentially normal S_2 state multiline EPR signal, but exhibit a substantially altered S_2-*minus*-S_1 FTIR difference spectrum. The intensities of the mutant EPR and FTIR difference spectra ($> 75\%$ compared to wild-type) are much greater than the O_2 signals and suggest that CP43-Glu354Gln PSII reaction centers are heterogeneous, with a minority fraction able to evolve O_2 with normal O_2 release kinetics and a majority fraction unable to advance beyond the S_2 or S_3 states. The S_2-*minus*-S_1 FTIR difference spectrum of CP43-Glu354Gln PSII particles is altered in both the symmetric and asymmetric carboxylate stretching regions, implying either that CP43-Glu354 is exquisitely sensitive to the increased charge that develops on the Mn_4Ca cluster during the S_1 to S_2 transition or that the CP43-Glu354Gln mutation changes the distribution of Mn(III) and Mn(IV) oxidation states within the Mn_4Ca cluster in the S_1 and/or S_2 states.

2391-Pos Fluorescence Lifetime Imaging of Chlamydomonas reinhardtii Following High Pressure Application

Yi-Chun Chen[1], Robert M. Clegg[2,3]

[1] *Department of Bioengineering, University of Illinois at Urbana-Champaign, Urbana, IL, USA,*
[2] *Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA,*
[3] *Center of Biophysics and Computational Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA.*

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Spinning-disk confocal fluorescence lifetime-resolved microscopy SPD-FLIM is used to measure changes in the spatial distribution of chlorophyll fluorescence within cells of the alga Chlamydomonas reinhardtii following the application of high hydrostatic pressure ($<$1100 bar). Spatial inhomogeneities in the fluorescence intensities and lifetimes are compared to that of cells at atmospheric condition. Upon pressure application, the fluorescence intensity initially increases. The maximum value of intensity, and the time that this maximum is reached (500–1000 seconds), depends on the pressure level. The fluorescence eventually decreases to a plateau value, which is the same for all pressures $>$500 bar. By measuring the spatial distribution of the fluorescence lifetime changes, SPD-FLIM provides information about the integrity of the photosynthesis system and structural changes in the chloroplasts.

2392-Pos Protein-Cofactor Interactions: Effects of a Single Amino Acid Change on the Redox Properties of Protein-Bound Cofactors

Nithya Srinivasan[1], Irina Karyagina[2], Dietmar Stehlik[2], John H. Golbeck[1]

[1] *Pennsylvania State University, University Park, PA, USA,*
[2] *Freie University, Berlin, Germany.*

Board B507

Many critical biological processes depend on electron transfer (ET) between protein-bound cofactors that are held at optimal distances by the protein matrix that modulates their redox properties. The approach described here assesses the effect of a single amino acid substitution on Photosystem I (PSI). PSI is a multisubunit pigment-protein complex that converts photons to chemical bond energy. It consists of the PsaA/PsaB heterodimer that harbors six chlorophylls, two phylloquinones, and one [4Fe-4S]-cluster, F_X. The phylloquinone in the ET chain oxidizes A_0^-, the primary electron acceptor and reduces F_X. The crystal structure indicates that the phylloquinone is H-bonded to $Leu722_{PsaA}$/$Leu706_{PsaB}$ and is pi-stacked with $Trp697_{PsaA}$/$Trp677_{PsaB}$. Apart from electrostatic considerations, these two features are deemed important in modulating its low redox potential (-770mV). Here, the H-bonded $L722_{PsaA}$ residue is replaced with a bulky Trp residue. Low temperature transient electron paramagnetic resonance experiments indicate that the orientation of phylloquinone is unaltered in the variant. However, the methyl hyperfine coupling (hfc) structure (quartet with relative intensities 1:3:3:1 centered near g_{yy} component of the g-tensor) is less pronounced, indicating a weakening or possible loss of the H-bond. Further, the hfc lines are temperature dependent, signifying an increase in the flexibility of the quinone in its binding pocket. High temperature studies show that the ET from the quinone to F_X is an order of magnitude faster in comparison to wild-type. Further, the preceding ET step from A_0^- to quinone remains unchanged. Thus, the quinone in the variant has a more negative midpoint potential than in native PSI. Temperature dependence studies indicate that the ET from the quinone to F_X has lower activation energy than in native PSI. Further experimentation will characterize the H-bond and map the distribution of quinone orientations.

Computational Methods - I

2393-Pos CHARMM-GUI: Graphical User Interface for CHARMM Users

Sunhwan Jo, Taehoon Kim, Wonpil Im

The University of Kansas, Lawrence, KS, USA.

Board B508

Molecular dynamics simulations of proteins have provided deeper insights into their functions and interactions with surrounding environments at the atomic level. However, writing input files for molecular dynamics simulation software can be challenging for some time, especially if the input files are involved with sophisticated tasks like solving PB (Poisson-Boltzman) Equation, solvating a protein in an implicit solvent, and building a realistic protein/membrane complex. The CHARMM-GUI (http://www.charmm-gui.org) website is a graphical user interface for molecular dynamics for biology scientists. Using this interface, a scientists can read and modify PDB from various sources and generates versatile input files from solvating a protein in an explicit or implicit solvent and solving PB (Poisson-Boltzman) Equation to compute solvent accessible surface to building a fully explicit protein-membrane complex. The interface also provides an easy access to set up the periodic boundary condition and environment for molecular dy-

namics. The input and output files generated while using the interface are freely available on the CHARMM-GUI website.

2394-Pos FRODA-MD: A Multiscale Method for Simulating Protein Dynamics

Craig C. Jolley, Daniel W. Farrell, Michael F. Thorpe

Arizona State University, Tempe, AZ, USA.

Board B509

The dynamics of proteins in solution involve a variety of processes on a wide range of time scales, from bond vibrations on the femtosecond scale to large domain motions on the millisecond scale. All simulation methods involve some degree of coarse-graining, and the coarseness with which the system is represented in a particular method largely determines the range of accessible time scales. There is a pressing need for multiscale methods that can integrate the effects of dynamical processes across a variety of time scales.

FRODA (Framework Rigidity Optimized Dynamical Algorithm) is a constrained geometric simulation method that models a protein as a number of rigid units which move relative to each other by unrestricted motion about dihedral bonds. A graph-theoretical algorithm called FIRST (Floppy Inclusion and Rigid Substructure Topography) is used to identify rigid regions in a protein based on the non-covalent interactions present. FRODA is able to quickly explore the conformational subspace consistent with a fixed set of non-covalent constraints, but is limited by an inability to break and form non-covalent constraints in a physically realistic fashion. To overcome this limitation, FRODA-MD is being developed as a multiscale method in which rapid phase-space exploration using FRODA is alternated with all-atom molecular dynamics simulations. This combination allows for rapid exploration of the subspace consistent with a given bond network, followed by modification of that bond network to open up new conformational subspaces. The eventual goal is to explore the large conformational changes associated with long timescales, without sacrificing the structural detail of all-atom methods.

2395-Pos The MARTINI Protein Force Field

D. Peter Tieleman[1], Luca Monticelli[1], Senthil K. Kandasamy[2], Xavier Periole[3], Ronald G. Larson[2], Siewert-Jan Marrink[3]

[1] *University of Calgary, Calgary, AB, Canada,*
[2] *University of Michigan, Ann Arbor, MI, USA,*
[3] *University of Groningen, Groningen, The Netherlands.*

Board B510

Many biologically interesting phenomena occur on a time scale that is too long to be studied by atomistic simulations. These phenomena include the dynamics of large proteins and self-assembly of biological materials. Coarse-grained (CG) molecular modelling allows computer simulations to be run on length and time scales that are 2–3 orders-of-magnitude larger compared to atomistic simulations, providing a bridge between the atomistic and the mesoscopic scale.

We developed a new CG model for proteins as an extension of the MARTINI force field for lipids. The model is computationally efficient and reproduces peptide-lipid interactions and the partitioning of amino acids and peptides in lipid bilayers. In order to validate the model, we calculated the potential of mean force for each amino acid as a function of its distance from the center of a DOPC lipid bilayer. Comparison with atomistic results shows good agreement. We used molecular dynamics simulations to study the partitioning, orientation and aggregation of the WALP23 peptide in lipid bilayers. WALP23 is a helical transmembrane peptide, and in the simulations unfolding is avoided using restraints based on the peptide conformation. Experimental data shows that WALP23 forms mainly monomers and small aggregates in lipid bilayers. We performed microsecond timescale simulations starting from 64 monomeric helices embedded in a DOPC bilayer. Partitioning and orientation are in good agreement with previous atomistic simulations, and the aggregation behavior is compatible with available experimental data. Reversible aggregation-disaggregation of the peptides is observed on the microsecond time scale.

2396-Pos A New Multi-resolution Procedure For Producing All-atom Equilibrium Ensembles Of Polypeptides: Combinatorial Decorating

Artem B. Mamonov, Daniel M. Zuckerman

Univ. of Pittsburgh Med. School, Pittsburgh, PA, USA.

Board B511

Building on our previous work with the “Resolution Exchange” (ResEx) algorithm, we introduce a new approach for generating all-atom equilibrium samples of polypeptides. We first prepare a “statistical rotamer library” for every side chain, which consists of all-atom configurations distributed according to the Boltzmann factor of the OPLS-AA forcefield. These libraries are only generated once, and will be made publicly available. To sample a specific polypeptide, we generate configurations in a “coarse-grained” model which, in our case, is an atomistic representation of the backbone (sampled inexpensively with the aid of pre-generated Ala, Gly, Pro libraries). We then “decorate” the backbone configurations by adding side chains, one at a time. At every stage, the partially decorated backbone ensemble is joined combinatorially with a corresponding side-chain library and re-weighted to conform to a Boltzmann factor distribution. The combination of pre-sampled components eliminates the need for expensive dynamical simulation - in contrast to ResEx. To assess our results, we perform a statistical efficiency analysis.

2397-Pos Sampling Molecular And Peptide Equilibrium Ensembles By Statistical Combination Of Molecular Fragments

Xin Q. Zhang, Artem B. Mamonov, Daniel M. Zuckerman
University of Pittsburgh, Pittsburgh, PA, USA.

Board B512

The simulation of equilibrium ensembles of small but flexible molecules by standard approaches is surprisingly expensive if high quality sampling is desired. We therefore employ a polymer-growth strategy and generate samples by combining molecular fragments in a non-dynamical, purely statistical way. In this initial study, we first combine two n-butanes to make n-octane, then combine two n-octanes to make n-cetane. We find good sampling efficiency compared to Langevin simulation. Beyond producing equilibrium samples of large size, our procedure simultaneously yields the associated absolute free energy values. We further demonstrate application of the method to peptides, where it has the potential to be extremely efficient by using pre-generated libraries of amino-acid configurations. The approach is currently limited to implicit solvent.

2398-Pos Effects Of Surface Water On Protein Dynamics Studied By A Novel Coarse-grained Normal Mode Approach

Lei Zhou, Steven A. Siegelbaum
Columbia university, HHMI, New York, NY, USA.

Board B513

Normal mode analysis (NMA) has received much attention as a direct approach to extract the collective motions of macromolecules. In this method the direction and amplitude of atomic motions are represented by the corresponding eigenvectors and eigenvalues of the Hessian matrix of the system potential energy function. However, the application of classical NMA based on the all-atom force field has been limited by the requirements of computer resources to store and diagonalize the very large Hessian matrix. Numerous coarse-grained approaches have been developed to improve the computational efficiency, including NMA based on an elastic network model (ENM) or block normal mode (BNM) method. Here we report the implementation of a novel coarse-grained normal mode approach (CGNM), based on a simple matrix partitioning scheme to separate the all-atom Hessian matrix into relevant and non-relevant parts, e.g. one group containing all C-α atoms and a second group containing all other atoms. Detailed chemical information imbedded in the non-C-α atoms is implicitly integrated into the dynamics of the C-α atoms. Using classical all-atom NMA as a reference, we found that the CGNM method generates more accurate results than do other coarse-grained approaches, including approaches based on ENM and BNM. Moreover, CGNM is able to incorporate contributions from non-C-α atoms, including explicitly treated surface water molecules, into the dynamics of C-α atoms, as indicated by the reduction in atomic fluctuations, the shift of vibrational modes to higher frequencies and the increase in the overlap with the eigenvector space from a principal component analysis of trajectories from molecular dynamics simulations. These results not only confirm the validity of the CGNM method but also point out the importance of incorporating surface structural water in studies of protein dynamics.

2399-Pos Failure of Replica-Exchange Dynamics to Generate the Boltzmann Ensemble

Scott C. Schmidler, Ben Cooke
Duke University, Durham, NC, USA.

Board B514

We demonstrate the practical implications of recently identified theoretical failures of replica exchange molecular dynamics (REMD). REMD simulations of polypeptides and other macromolecules have become increasingly popular due to their ability to cross large energy barriers. However, failure of isothermal molecular dynamics methods and associated numerical schemes to be either ergodic or invariant implies that phase space is not fully explored even in the limit of an infinitely long simulation. As a result, the simulation may not converge to the desired equilibrium Boltzmann ensemble. We demonstrate these failures of REMD algorithms on small but illuminating examples: a mixture of Gaussian distributions (simple harmonic oscillator dynamics) exhibiting two energy wells, and the Alanine dipeptide. Examination of the resulting phase plots and equilibrium configuration densities indicates significant errors in the ensemble generated by REMD simulation. We describe a simple modification to address these failures based on a stochastic hybrid Monte Carlo correction.

2400-Pos Large-scale Molecular Simulations In Fluids And Biosystms: Enhanced Sampling, Accelerated Molecular Dynamics And Coarse-grained Modeling

Xin Zhou
Los Alamos National Laboratory, Los Alamos, NM, USA.

Board B515

Atomic molecular simulations are standard tools to understand fluids and biosystems. The fundamental limitation of the simulations is that the accessible time and spatial scales are too small to study many interesting macroscopic phenomena and processes. We present some new methods, including the rho-ensemble sampling to overcome quasiergodicities of systems at low temperature, the accelerated molecular dynamics in entropic-dominated systems based on time-compression transformation and path-space coarse-graining techniques to get slow dynamics, and bridging different-level coarse-graining models. The methods are applied

in simple fluids, glasses and biopolymers to simulate the large-scale equilibrium and dynamical (or kinetic) properties but keeping the needed atomic details.

2401-Pos Coarse Grained Molecular Dynamics Simulations Of Protein-lipid Interactions With Designed Transmembrane Peptides

Bram van Hoof[1], Peter Spijker[1], Nagarajan Vaidehi[2], Peter A.J. Hilbers[1]

[1] *Technische Universiteit Eindhoven, Eindhoven, The Netherlands,*
[2] *City of Hope, Duarte, CA, USA.*

Board B516

Interaction of peptides with membrane leads to structural rearrangements in both the membrane and the peptides. Factors that influence these structural rearrangements are the length and nature of the transmembrane (TM) peptide sequence, the composition, and the nature of the lipids in the membrane. Understanding of the interaction of peptides with lipids involved in these structural rearrangements, would help uncover the organization in cellular membranes. The time scale of the insertion of peptides into membranes causing structural rearrangements is longer than accessible by conventional molecular dynamics simulations. We have developed a coarse grained forcefield model for TM proteins. This model reduces the simulation time considerably, and allows focus on the overall behavior of these proteins in lipid bilayers.

Based on the coarse grained model by Markvoort *et al.* [1], we developed a two particle per amino acid model for the peptide: one for the backbone, one for the side chain. This model has been used to study the tilt angles of WALP and KALP repeat peptides of various lengths in DLPC, DPPC and DEPC. The tilt angles correlate well with experimental measurements using FTIR spectroscopy. We also find the effect of the hydrophobic mismatch manifested as adapting of the thickness of the lipid bilayer. This also agrees with the experimental results. Thus these peptides allow a systematic study of the effect of hydrophobic mismatch between a lipid bilayer and a TM peptide, on the incorporation of a peptide into the membrane, the membrane thickness, and the peptide tilt. Coarse grained simulations have also been performed on TM peptides that show varying degree of incorporation into lipid bilayers. The results of these simulations will be presented.

References

[1]. A.J. Markvoort, et al., J. Phys. Chem. B, *109*: 22649–22654 (2005)

2402-Pos Predicting Tilt Angles and Free Energy Profiles for a Transmembrane Helix: A Comparative Study of Two Implicit-Solvent Lipid Models

Aaron T. Frank, Ioan Andricioaei

University of Michigan, Ann Arbor, MI, USA.

Board B517

Free energy profiles along predefined coordinates can be efficiently calculated using umbrella simulations. Such simulations generate flat distributions but they are difficult to converge because of very long equilibration times of the solvent in explicit representation, particularly when in lipid bilayers are involved. Two implicit lipid membrane models are here applied in combination with the umbrella sampling strategy to the simulation of the transmembrane (TM) helical segment from virus protein U (Vpu). The models are used to study both orientation and energetics of this α - helical peptide as a function of hydrophobic mismatch. We observe that increasing the degree of positive hydrophobic mismatch increased the tilt angle of Vpu. These findings agree well with experiment, and as such validate the solvation models used in this project.

2403-Pos Understanding of Influence of Hydrophobic Mismatch on Transmembrane Helix Tilting by Potential of Mean Force Calculations as a Function of Tilt Angle

Jinhyuk Lee, Wonpil Im

Univ. of Kansas, Lawrence, KS, USA.

Board B518

The hydrophobic match between transmembrane domains and the lipid bilayer has been recognized as a central feature in protein-lipid interactions and bilayer regulation of membrane protein functions. Traditionally, people have envisioned that a transmembrane helix may tilt or kink in order to overcome unfavorable interactions arising from a hydrophobic mismatch. To determine the microscopic forces governing the helix tilting in membranes, we have calculated the potential of mean force as a function of tilt angle of various transmembrane model peptides in membranes. The total potential of mean force and its decomposition reveal that the helix tiling in membranes is governed by interplay between an intrinsic entropy contribution arising from the helix precession around the membrane normal and the sequence- and length-specific helix-lipid interactions. The role of specific helix-lipid interactions in the helix tilting is presented and discussed in terms of potentials of mean force and their decompositions of transmembrane helices with different hydrophobic lengths and different anchoring residues at the membrane interface, and in lipid bilayers with different hydrophobic thickness. Furthermore, the efficacy of the new restraint potential for helix rotation around the helical principal axis to identify the minimum helix rotation angle will be presented.

2404-Pos Limits In Application Of Linear Elasticity Theory In Characterization Of Alpha-helices And Filamentous Proteins

Sirish K. Lakkaraju, Wonmuk Hwang

Texas A&M University, College Station, TX, USA.

Board B519

Elasticity of biofilaments is typically described by linear response to local bending, with a bending stiffness that sets the persistence length as a fundamental scale describing their flexibility. However, most biofilaments possess attractive interactions that are not screened in physiological conditions and cause bundling. We investigated the effect of non-bonded attractive interactions on the elasticity of a single filament, with alpha-helices as examples. Normal mode analysis (NMA) revealed that, below the length of about 70 residues, the helix exhibits a linear elastic behavior with the bending stiffness on the order of $3 \times 10^{-28} Nm^2$. These values were relatively insensitive to the amino acid sequence, as the filament elasticity is governed mainly by the elastic core formed by backbone hydrogen bonds rather than by side chain interactions. However, beyond this size, lowest normal modes disappeared, suggesting that the linear response deteriorates as longer helices are considered. To confirm that the breakdown of linear elasticity is caused by non-bonded attractions, we tested a beads-on-chain model using NMA. When only the bending energy between two adjacent bonds is considered, the system remained linear elastic at all lengths tested. However, introduction of non-bonded attraction between beads resulted in disappearance of lowest normal modes beyond certain lengths that depend on the strength of the attractive potential. We explain this in terms of the buckling instability due to the intrinsic attractive force in the filament, which is well-described by a modified wave equation for an elastic rod under an internal force. The calculated critical buckling length for an alpha-helix is about 65nm, which is temperature-independent. These results suggest that the critical buckling length could be a more important length scale in cases when it is shorter than the persistence length.

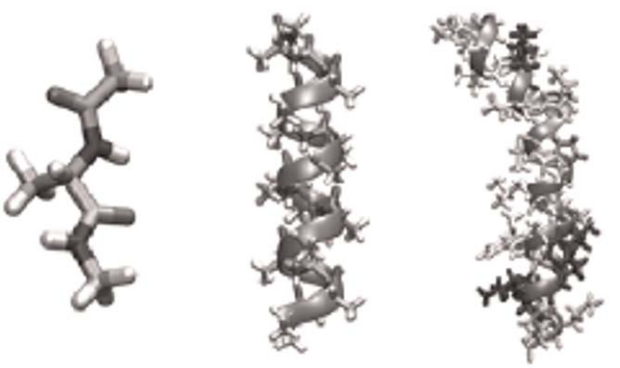
2405-Pos Conformational Sampling of Peptides in Cellular Environments

Seiichiro Tanizaki, Jacob Clifford, Brian D. Connelly, Michael Feig

Michigan State University, East Lansing, MI, USA.

Board B520

Biological systems provide a complex environment that can be understood in terms of its dielectric properties. High concentrations of macromolecules and co-solvents effectively reduce the dielectric constant of cellular environments. In order to examine how conformational sampling may be affected in such environments, the conformational preference of alanine dipeptide, poly-alanine, and melittin in different dielectric environments is studied with computer simulations based on recently developed generalized Born methodology. Results from these simulations suggest that extended conformations are favored over α-helical conformations at the dipeptide level at and below dielectric constants of 5–10. Lower-dielectric environments significantly stabilize helical structures in poly-alanine at and below ε=20. In melittin, different dielectric environments shift the equilibrium between two main conformations: a nearly fully extended helix that is most stable in low dielectrics and a compact, V-shaped conformation consisting of two helices that is preferred in higher dielectric environments. An additional conformation is found at intermediate dielectric constants. Good agreement with previous studies of different peptides in specific, less-polar solvent environments, suggest that helix stabilization and shifts in conformational preferences in such environments are primarily due to a reduced dielectric environment rather than specific molecular details.



2406-Pos Using Molecular Dynamics Simulations to Interpret the Spectral Signatures Obtained from Two-Dimensional Infrared Spectroscopy of a Helical Octapeptide

Neelanjana Sengupta, Hioraki Maekawa, Wei Zhuang, Nien-Hui Ge, Shaul Mukamel, Douglas J. Tobias

University of California, Irvine, Irvine, CA, USA.

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We present structural analysis and 2DIR spectra of the amide-I vibrational mode of a C_α-methylated peptide, Z-[L-(αMe)Val]$_8$-O*t*Bu, solvated in deuterated chloroform. Spectral calculations from molecular dynamics simulation trajectories, performed with a modified CHARMM force field for the peptide and an all-atom model of chloroform from AMBER force field, are compared with 2DIR experiments in the $<\pi/4, -\pi/4, Y, Z>$ polarization configuration (Maekawa et al., JPCB 2006). Experiments with rephasing and non-rephasing pulse sequences on this system have revealed a doublet of peaks and a single major peak, respectively. Conformations obtained from simulation trajectories are used to calculate spectra using the vibrational exciton model and the sum-over-states formulation. The site energies are calculated by two electrostatic models: Mukamel's DFT map as implemented in the SPECTRON package (Zhuang et al., JPCB 2006) and Cho's potential model (Ham et al., JPCB 2003). Structural determination of simulated conformers is done by hydrogen bond analysis as well as by dihedral angle analysis of each residue. It is seen that unrestrained simulations drive the system to unexpected regions in the Ramachandran space, giving rise to extra features in the 2D spectra. Small backbone positional restraints are found to be useful in obtaining trajectories that reproduce the experimental rephasing and non-rephasing spec-

tra. The spectra calculated under the double-crossed polarization are more sensitive to subtle structural changes than those under the parallel polarization. Greatest agreement between experimental and calculated spectra occurs for largely 3_{10}-like conformations. Deficits of the current force fields in structure determination of unnatural peptides are pointed out. This study correlates the 2DIR spectral features to details of helical secondary structure, while comparing results obtained from different methods of spectral calculation with experiments.

2407-Pos Computer Modeling Of Protein's Mechanical Unfolding: Transition State, Unfolding Pathway, And Protein Design

Gang Feng, Ognjen Perisic, Anuradha Mittal, Hui Lu

Bioinformatics, Department of Bioengineering, University of Illinois at Chicago, Chicago, IL, USA.

Board B522

Force-induced protein unfolding and unbinding are involved in many biological processes and are crucial to mechanical signaling. Previously we have studied extensively the forced unfolding of several mechanical proteins such as titin, fibronectin, and ubiquitin. A local stability mechanism was proposed and validated which states a shear pulling on a beta sheet contribute to the strong mechanical resistance. Recent single molecule microscopy experiments started going beyond the standard pulling mechanical proteins. Inspired and complement to these experiments, we are using steered molecular dynamics to obtain deeper understanding of mechanical resistance. Comparing the unfolding of protein in various solvent, we have found the mechanical transition state bridges through solvent molecules. Thus mechanical protein goes through different transition state in various solutions, which reflected in the different mechanical stability. Also studied was a protein which has a designed topology, Top7, which is symmetric. Computer simulation suggested unfolding from N termini part is favored. However, when this pathway is blocked, the C-termini pathway will be activated and causes higher mechanical resistance. Thus we have proposed a new way for tuning mechanical stability. All the simulations have been validated by our experimental collaborators.

2408-Pos The Role of the Disulfide Bridge on the Conformational Relaxation of Cu, Zn Superoxide Dismutase Upon Loss of Metals Studied by Molecular Dynamics

Shawn M. Hamm, Alfredo E. Cardenas

University of South Florida, Tampa, FL, USA.

Board B523

Cu, Zn Superoxide Dismutase (SOD) is an essential cytosolic, antioxidant enzyme found in eurokaryotic cells. This 32kDa, homodimer contains one catalytic copper and one structural zinc ion per monomer. Misfolding and subsequent aggregation of SOD has been linked to neurodegenerative disorders such as Amyotrophic Lateral Sclerosis (ALS). Experimental evidence suggests loss of zinc and reduction of the intra-subunit disulfide bridge are correlated with aggregation and toxic gain of function. The conformational relaxation of a bridge-reduced, copper-depleted, mutant structure was studied via a 20ns molecular dynamics in explicit solvent after Zn ion removal. The dynamics of this mutated, bridge-reduced variant was then compared to a similar, but shorter (10ns) simulation of apo-SOD (bridge present). These two simulations were then analyzed via principal component analysis. It was found that the region with the most difference (50–72) contains one of the Cysteine (C57) residues that form the disulfide bridge. The backbone dihedral angles of these residues were analyzed as a function of time. These residues unwound, "flipped over" and wound back up, leading to a partial unfolding when this bridge was broken. The presence of this increased flexibility surrounding C57 suggests the disulfide bridge is essential at stabilizing loop IV.

2409-Pos A Replica-exchange Molecular Dynamics Study On Structural Changes Of The Cytoplasmic Domain Of Phospholamban By Phosphorylation At Ser16

Yuji Sugita[1,2], Naoyuki Miyashita[3], Takao Yoda[4], Mitsunori Ikeguchi[5], Chikashi Toyoshima[3]

[1] *RIKEN, Wako, Japan,*
[2] *JST, CREST, Honcho Kawaguchi, Japan,*
[3] *the University of Tokyo, Tokyo, Japan,*
[4] *Nagahama Bio University, Nagahama, Japan,*
[5] *Yokohama City University, Yokohama, Japan.*

Board B524

Phospholamban (PLN) is a 52-residue integral membrane protein that regulates the activity of the sarcoplasmic reticulum calcium pump in cardiac muscle. Its inhibitory action is relieved when PLN is phosphorylated at Ser16 by cAMP-dependent protein kinase (PKA). To computationally explore all possible conformations of the phosphorylated form, and thereby to understand the structural effects of phosphorylation, replica-exchange molecular dynamics (REMD) was applied to the cytoplasmic domain that includes Ser16. The simulations showed that

(i) without phosphorylation, the region from Lys3 to Ser16 takes all α-helical conformations;
(ii) when phosphorylated, the α-helix is partially unwound in the C-terminal part (from Ser10 to Ala15) resulting in less extended conformations;
(iii) the phosphate at Ser16 forms salt bridges with Arg9, Arg13, and/or Arg14;
(iv) the salt bridges with Arg13 and Arg14 distort the α-helix and induce unwinding of the C-terminal part.

The distortions caused by the salt bridges involving the phosphate at Ser16 readily explain the relief of the inhibitory effect of PLN by phosphorylation, as they will substantially reduce the population of all helical conformations, which are presumably required for the

binding to the calcium pump. This will also be the mechanism for releasing the phosphorylated PLN from kinase.

2410-Pos Influence of Phosphorylation on Conformational Change of Phospholamban Cytoplasmic Domain and Its Interactions with Membranes: Molecular Dynamics Studies by Potential of Mean Force Calculations

Taehoon Kim, Jinhyuk Lee, Wonpil Im

The University of Kansas, Lawrence, KS, USA.

Board B525

Phospholamban (PLB) is an integral membrane protein of 52 amino acids that consists of amphiphatic cytoplasmic (Met1-Ser16) and transmembrane (Gln22-Leu52) domains. PLB modulates the intercellular calcium levels by regulating the activity of the Sarco(Endo) plasmic Reticulum (SR) Calcium ATP-ase (SERCA) of cardiac, slow twitch and smooth muscles. Binding of PLB to SERCA inhibits the SERCA activity and thus decreases the calcium influx into SR, which results in reduction of the cardiac relaxation rate. A conformational change of PLB upon phophorylation of its Ser16 by protein kinase A causes PLB to dissociate from SERCA. Recent experiments have illustrated the dynamic nature of PLB cytoplasmic domain upon phosphorylation; the population of unphosphorylated PLB cytoplasmic domain that interacts with membranes is about 84%, but its population reduces by 20% when phosphorylated. However, it is not clearly understood why such different conformational preferences occur depending on phosphorylation. To characterize the underlying driving forces that govern the dynamics and conformational change of PLB cytoplasmic domain upon phosphorylation, we have calculated the potentials of mean force (PMFs) as a function of tilt angle of PLB cytoplasmic domain in a POPC membrane with and without phosphorylation. Detailed information on energetics of conformational change of PLB cytoplasmic domain as well as its interactions with membranes will be presented.

2411-Pos Structural Evaluation of the Effects of Disulfide Bond Eliminations on Scorpion Toxin λ-Hefutoxin1 from *Heterometrus fulvipes* by Molecular Dynamics Simulations

Fatemeh Jazayeri, Maryam Heydari, Maryam Ghobeh, Mehriar Amininasab, Elahe Elahi

Department of Cell and Molecular Biology, Faculty of Science, University of Tehran, Tehran, Iran (Islamic Republic of).

Board B526

κ-Hefutoxin1, from the venom of scorpion *Heterometrus fulvipes*, is a member of the new class of weak potassium channel toxins (κ-KTx) with unique spatial fold lacking any β-sheets consisting of two parallel helices linked by two disulfide bridges between the four existing cysteine residues.

For this study, the disulfide bonds in κ-Hefutoxin1 were eliminated in three series of the following mutations: Cys4Ser and Cys22Ser in the first series; Cys8Ser and Cys18Ser in the second series; and finally all four cysteines replaced by serine in the third series of substitutions. The molecular dynamics simulation of the wild and mutant types of κ-Hefutoxin1 were performed in explicit water and 33% (v/v) TFE solutions for sufficiently long simulation time of 25 ns to be able to reveal the structural effects of the mutations.

Analysis of simulation trajectories allow to study the structural effects of disulfide bonds on the spatial integrity of κ-Hefutoxin1 in water and TFE solutions.

2412-Pos The peripheral binding of Pr3 to lipid bilayers: A Molecular Dynamics study

Torben Broemstrup

University of Bergen, Bergen, Norway.

Board B527

Proteinase3 (Pr3) is a serine protease of the neutrophils involved in inflammation processes and its membrane expression is a risk factor for chronic inflammatory diseases such as vasculitis or emphysema and is thus a potential drug target.

In addition, biophysical experiments showed different in vitro binding affinities for Pr3 to membranes of different lipid mixtures. Whereas negative lipids are increasing the general binding affinity of most peripheral membrane proteins, the strongest binding affinity for Pr3 was observed for a one to one mixture of DMPC (zwitterionic) and DMPG (negative) lipids. Positively charged basic residues and their electrostatic interactions with the polar head groups mainly drive the binding. In addition, the hydrophobic amino acids anchor into the hydrocarbon region, which provides a small but significant hydrophobic contribution to the binding.

Previous work in our group identified a potential membrane-binding site for Pr3 using an implicit membrane model and produced results that were in good agreement with the biophysical observed.

In order to design compounds for the inhibition of the membrane expression of Pr3 we need a detailed picture of the membrane interactions.

Therefore, we ran Molecular Dynamics simulations with explicit membrane models to obtain an all atom model for the binding of Pr3 to lipid bilayers.

A preliminary step, was testing the effect of applying different surface tensions parameters to membrane models (DMPC / DMPG mixtures) in order to reproduce experimental observables. Inserting Pr3 into these membrane models to run MD-simulations gave new insight into the membrane-binding of Pr3. These results will enable the rational design of drugs to efficiently and selectively inhibit the expression of Pr3 on biological membranes.

2413-Pos On the Nature of Antimicrobial Activity: A Model for Protegrin-1

Allison A. Langham, Abdallah Sayyed Ahmad, Yiannis N. Kaznessis

University of Minnesota, Minneapolis, MN, USA.

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Though antimicrobial peptides have been studied for more than two decades as possible substitutes for traditional antibiotic drugs, their mechanism of action is still not fully understood. We have performed over 150ns of simulation of a protegrin-1 (PG-1) pore in a lipid bilayer composed of plamitoyloleoylphosphatidylethanolamine (POPE) and palmitoyloleoylphosphatidylglycerol (POPG) lipids meant to mimic the inner membrane of a bacterial cell. The simulations improve on a model of an octomeric pore proposed from NMR experiments. From the results of the simulation, we determine that the pore more closely follows the barrel-stave model than the toroidal model for insertion into the bilayer. We explore the movement of ions through the pore in detail. The pore allows negatively charged chloride ions to pass through at an average rate of one ion per two nanoseconds. We observe only two events of sodium ions crossing through the pore. Potential of mean for was calculated for the water and both ion types and it is determined that the chloride ions move through the pore as easily as the water molecules. We explore the potential for PG-1 to kill cells through osmotic lysis or destabilization of transmembrane potential.

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2414-Pos Potential of Mean Force and Binding Free Energy Calculations for Antimicrobial Peptide LactoferricinB with Membranes

Victor M. Vivcharuk[1], Bruno Tomberli[2], Igor Tolokh[1], Chris Gray[1]

[1] *University of Guelph, Guelph, ON, Canada,*
[2] *Brandon University, Brandon, MB, Canada.*

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Microscopic molecular dynamics (MD) simulations have been used to study the interactions of anionic palmitoyloleoylphosphatidylglycerol (POPG) and zwitterionic phosphatidylcholine (POPC) bilayers with the cationic peptide lactoferricin B (LFCinB). The interactions of LfcinB with POPG and POPC are used as a model system for studying membrane peptide selectivity and understanding the mechanism leading to peptide-induced membrane disruption.

To calculate the potential of mean force (PMF) for the peptide versus distance between LFCinB and membrane centers of mass a combination of constrained MD and thermodynamic integration techniques was used. We find for the LFCinB-POPC system that the PMF has the minimum energy profile where the peptide backbone is parallel to the membrane and the side facing the membrane contains most of the aromatic residues. For LFCinB-POPG the most energetically favorable orientation of LFCinB occurs when most of the basic residues face the membrane.

A simplified method for relating the PMF to the LFCinB-bilayer binding free energy was developed and used to calculate a free energy of binding of −6.64 kcal/mol for POPG and −1.05 kcal/mol for POPC.

Decomposition of the force acting on the LFCinB into system components (membrane, water, ions) and type of the interaction (electrostatic, van der Waals) provides insight into the nature of the contributions to the PMF. The calculation of the forces between different components of the system shows that the only source of attractive force acting on the LFCinB at very short distances is the direct membrane-peptide electrostatic interaction.

The simulations support the hypothesis that some cationic peptides bind to an anionic membrane by replacing cationic ions which normally function as cationic bridges between adjacent phosphates.

2415-Pos A Multi-Scale Approach to Computing a 1-D Potential of Mean Force Profile applied to a Kv Channel Toxin/Phospholipid Bilayer System

Chze Ling Wee, David Gavaghan, Mark Sansom

University of Oxford, Oxford, United Kingdom.

Board B530

Computing accurate free energies via molecular simulations remain a key challenge due to the large computational requirements necessary for sufficient sampling and convergence. Recent coarse-grained (CG) approaches allow for the simulation of significantly larger membrane/protein systems over longer periods of time. Such models have been successful at reproducing qualitative behaviour to a large degree when applied to membrane/protein systems. However, their ability to reproduce thermodynamic properties remains uncertain. We have recently used a CG protein and lipid model to compute the 1-D potential of mean force (PMF)/free energy profile of placing a voltage-gated potassium (Kv) channel gating-modifier toxin (VSTx1, which binds the voltage-sensors of the archaebacterial Kv channel KvAP) at different depths in a phospholipid bilayer. Here, we utilize information gained from the CG simulations to initiate corresponding atomistic (AT) simulations. Such coupling allows for the avoidance of local minima in the potential energy landscape which is unlikely to occur over finite AT simulation timescales. We show that the free energy profiles obtained from the CG and AT simulations are comparable. Future efforts should therefore focus on more intelligent coupling approaches across multiple levels of granularity.

2416-Pos Calculating Potentials of Mean Force for Large Biomolecules from Nonequilibrium Processes: Determination of Light Harvesting Complexes 2 Ring Sizes

Lorant Janosi[1,2], Harindar S. Keer[3], Ioan Kosztin[1], Thorsten Ritz[4]

[1] *Dept. of Physics and Astronomy, University of Missouri, Columbia, Columbia, MO, USA,*
[2] *Dept. of Chemical and Biomolecular Engineering, University of Houston, Houston, TX, USA,*
[3] *Dept. of Chemistry, University of California, Irvine, Irvine, CA, USA,*
[4] *Dept. of Physics and Astronomy, University of California, Irvine, Irvine, CA, USA.*

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Recently, several non-equilibrium methods, based on or related to the Jarzynski equality (JE), have been proposed for calculating potentials of mean force (PMFs) along a given reaction coordinate. However, most of these methods were applied to biomolecules of relatively small sizes, where sampling of many trajectories is attainable. In general, JE based methods fail when only a small number of trajectories can be sampled due to the lack of sampling of the extremely rare paths with negative dissipation work. To overcome this problem, the FR method [J. Chem. Phys. *124*, 064106 (2006)] was proposed for calculating PMFs from a small number of fast SMD pulling in both forward (F) and time reverse (R) directions. The FR method was developed by employing the Crooks transient fluctuation theorem and the stiff-spring approximation. This method is efficient and simple as both the PMF and the underlying diffusion coefficient can be expressed in terms of the mean work during the F and R pullings. To demonstrate the applicability of the FR method to generate PMFs efficiently for larger biomolecular systems, we describe here a methodology based on the FR method for determining the ring sizes of Light Harvesting Complexes 2 (LH2). Membrane embedded LH2 complexes of purple bacteria are found in octameric or nonameric form despite remarkable similarities among tertiary structure of monomers of LH2 from different purple bacteria. For the purpose of validation and evaluation of predictive power of the FR method, we apply developed methodology to predict ring sizes of LH2 complexes of Rhodospirillum (Rs.) molischianum (known, 8), Rhodopseudomonas (Rps.) acidophila (known, 9) and X (unknown, structure provided by an experimental group), given only the monomeric structure of complexes.

Computational Methods - II

2417-Pos Fully Flexible Four Site Polarizable Water Model With A Dynamic Extended Charge

Pradip K. Biswas[1], Bernard Brooks[2]

[1] *Tougaloo College, Tougaloo, MS, USA,*
[2] *NHLBI/NIH, Bethesda, MD, USA.*

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Polarizable water model and its extension to computational studies of biomolecular systems have been a goal of active research for quite sometime [1–3; *and references therein*]. The polarizability aspect is quite important to obtain the critical binding information of drug molecules to protein active sites and to find the change in the reactivity of proteins and DNA's with substrates. The recent focus on dedicated hardware and parallel algorithm for molecular dynamics [4], has eased the computational restriction for the use of a polarizable model. However, appropriate modeling still remains a challenge. The modeling of electronic charge redistribution, using a point-dipole or fluctuating charge model, though provide the number, not necessarily provide a satisfactory charge re-distribution which is essential for critical binding or reactivity studies.

We worked out a four site fully flexible water model where a Gaussian extended charge is harmonically attached to the electronegative oxygen atom and it is also made visible to the hydrogen atoms using harmonic bonds so that it can respond to both intramolecular and inter-molecular changes. This model is optimally designed for use with self-consistent flexible constraints where both the geometry and the position of the fourth center are simultaneously optimized. This allows for a fully flexible water model without the associated problems of non-physical high-frequency heat capacity [5]. Details of the model, optimization procedure, and results will be reported at the conference.

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2418-Pos Non van der Waals treatment of hydrophobic solubilities

Dilip Asthagiri[1], Safir Merchant[1], Henry Ashbaugh[2], Michael Paulaitis[3], Lawrence Pratt[4]

[1] *Johns Hopkins University, Baltimore, MD, USA,*
[2] *Tulane University, New Orleans, LA, USA,*
[3] *Ohio State University, Columbus, OH, USA,*
[4] *Los Alamos National Laboratory, Los Alamos, NM, USA.*

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A quasi-chemical theory implemented on the basis of molecular simulation is derived and tested for the hydrophobic hydration of CF_4(aq). The theory formulated here identifies chemical contributions to the hydration that naturally arise from chemical contributions defined by quasi-chemical theory and fluctuation contributions analogous to Debye-Huckel or random phase approximations. As judged by the size of the fluctuation contribution, the resulting Gaussian statistical thermodynamic model is physically reliable in these applications. The specific results here confirm that unfavorable tails of binding energy distributions of the solute with water reflect few-body close solute-water encounters. The water near-neighbors are pushed by the medium into unfavorable interactions